Abstract

Background:

Delayed postpolypectomy bleeding occurs more frequently after hot resection than after cold resection.

Objective:

To elucidate the underlying mechanism, we performed a histological comparison of tissue after cold and hot snare resections.

Design:

This is a prospective study, registered in the University Hospital Medical Information Network (UMIN000020104).

Setting:

Aizu Medical Center, Fukushima Medical University, Japan

Patients:

Fifteen patients scheduled to undergo resection of colorectal cancer were enrolled.

Intervention:

On the day before surgery, two mucosal resections (hot and cold) of normal mucosa were performed on each patient using the same snare without saline

injection. The difference was only the application of electrocautery or not. Resection sites were placed close to the cancer, to be included in the surgical specimen.

Main Outcome Measures:

The primary outcome measure was the depth of destruction. Secondary outcome measures included the width of destruction, depth of the remaining submucosa and number of vessels remaining at the resection sites. The number and diameter of vessels in undamaged submucosa were also evaluated.

Results:

All cold resections were limited to the shallow submucosa, while 60% of hot resections advanced to the deep submucosa and 20% to the muscularis propria ($p<0.001$). There was no significant difference in the width of destruction. The number of remaining large vessels after hot resections trended toward fewer ($p=0.15$) with a decreased depth of remaining submucosa ($p=0.007$). In the deep submucosa, the vessel diameter was larger ($p<0.001$) and the number of large vessels was greater ($p=0.018$).

Limitations:

Histological assessment was not blinded to the two reviewers. Normal mucosa

was used instead of adenomatous tissue.

Conclusions:

Hot resection caused damage to deeper layers involving more large vessels. This may explain the mechanism for the reduced incidence of hemorrhage after cold snare polypectomy.

Keywords: cold snare polypectomy, hot snare polypectomy, electrocautery, procedure-related complications, delayed bleeding

Abbreviations

SM: submucosa

MP: muscularis propria

Introduction

Colonoscopic resection of adenomatous polyps reduces colorectal cancer incidence and mortality, [1-3] and is widely accepted as a standard procedure to eradicate pre-malignant lesions. Polypectomy using electrocautery, known as a hot polypectomy, has the added effect of tissue ablation to promote complete resection and hemostasis, but there is an increased risk of procedure-related complications such as bleeding and perforation. [4-8] There are numerous case series describing significant hemorrhage after hot resections, which have resulted in the need for surgical rescue or even death. [9-11] In a 1988 survey, 516 American Society for Gastrointestinal Endoscopy members performed 13,081 hot biopsy forceps resections (one type of hot resection), and 16% reported patients with significant complications such as bleeding, perforation, post-coagulation syndrome, or death [12].

In 1992, cold snare polypectomy, the mechanical resection of colorectal polyp without electrocautery, was advocated due to less frequent complications;[13] this attracted the attention of colonoscopists. Cold snare polypectomy shortens the procedure time [14] and reduces the risk of complications, especially delayed bleeding[15-19]. Cold snare polypectomy is

considered the optimal method for resection of diminutive polyps in western countries. In a 2014 survey of 244 Gastroenterological Society of Australia members, 75% performed cold snare polypectomy for polyps ≤3 mm and 49% for polyps ≤5mm.[20] In Japan, however, the use of cold snare polypectomy is still limited.[19]

It has been suggested that delayed bleeding after hot snare polypectomy is due to cauterization-related thermal injury of submucosal vessels.[4, 21] In a porcine model, hot resection caused submucosal necrosis and inflammation of the deeper layer, including the muscularis propria, [22] which indicates that thermal injury increases the risk of vessel damage and subsequent bleeding in the colon. One report described that hot resection of colonic lesions leads to a similar histological change in surgical specimens, but there was no direct comparison with cold resections.[23] The histological architecture of the submucosa (SM) has not previously been investigated after either cold or hot resections. Therefore, we performed this study to compare the histology of the submucosal vessels and stroma after cold and hot resections.

The distribution, number and diameter of vessels in the SM has not been previously described. Detailed examination may explain the occurrence of

less frequent delayed bleeding after cold resections. We also investigated the histology of the vessels in the normal SM.

The aim of this study was to clarify the underlying mechanism in order to explain why delayed bleeding occurs less frequently after cold resection than after hot resection. Elucidation of the underlying mechanism may help to establish cold snare polypectomy as a standard procedure not only for diminutive polyps, but also for larger ones.

Materials and Methods

Study design/setting

The study protocol followed the ethical guidelines of the Helsinki Declaration, and was approved by the Institutional Review Board at Fukushima Medical University. The study protocol was registered in the University Hospital Medical Information Network (UMIN000020104) on December 10, 2015. Written informed consent was obtained from all patients. The STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines were followed in reporting this study.

Patients

From December 2015 to November 2016, 15 patients who were scheduled to undergo colorectal resection for cancer were prospectively enrolled. Patients who were American Society of Anesthesiologists Class III or IV, patients with hemorrhagic diatheses and patients receiving antithrombotic therapy were excluded from the study.

Endoscopic Resection

Patients were admitted to the hospital two days before surgery. In the evening, patients were given a mechanical bowel preparation regimen of 7.5 mg sodium picosulfate hydrate (Laxoberon®: Teijin Pharma, Ltd., Tokyo, Japan). On the day before surgery, patients drank 1L of polyethylene glycol lavage plus ascorbic acid solution (Moviprep®: EA Pharma, Tokyo, Japan. each liter contains 100.0g macrogol 4000, 7.5g sodium sulfate, 2.7g sodium chloride, 1.0g potassium chloride, 4.7g ascorbic acid, 5.9g sodium ascorbate, and lemon flavoring) and 0.5 L of water four hours before colonoscopy. Colonoscopic procedures were performed by five endoscopists (D.N., N.I., S.E., K.U. and M.A.) with the experience of more than 1000 endoscopic resections.

Two different types of mucosal resections (hot and cold), using the same snare wire (13mm Captivator™, Boston Scientific, Japan), of normal mucosa were performed in the same patient without saline injection, resulting in an approximately 1cm^2 mucosal defect. The technique for endoscopic resection without saline injection was followed, as described by Pattullo and colleagues.[24] The only difference between hot and cold snare resection was whether or not electrocautery was used. For hot snare resections, an ERBE VIO300™ (Amco, Tokyo, Japan) was used in the Endocut Q mode with effect 2, cut duration 3, and cut interval 3. To include the endoscopic resection sites in the surgical specimens, the sites were selected close to the distal side of the cancer or scars after a previous endoscopic removal (Figure 1), and on the proximal side in patients with rectal lesions. The resection sites were aligned longitudinally, rather than axially, and were selected in a random fashion. Bowel resection with lymphadenectomy was carried out the following day in the routine manner.

Preparation of Surgical Specimens

In order to avoid injury at the endoscopic resection sites, surgical specimens were longitudinally opened along the side opposite the lesion and

pinned on a corkboard for fixation in 15% formalin for 24-48 hours. Discrimination between hot and cold resection sites was made based on the colonoscopic reports. The endoscopic resection sites were cut axially (not longitudinally) into 2mm-thick sections, and these sections were totally embedded in paraffin. The slices were cut into 2μm section using a microtome and stained with hematoxylin-eosin and Elastic Masson, according to the standard protocols. Regardless of whether it was cancer or scar, lesions were processed using the same protocol.

Histological Assessment

Under the guidance of a pathologist (H.H.), the histology was reviewed by two of the authors (D.T., D.N.) on a computer display using cellSens^{TM} (Olympus, Tokyo, Japan) software. Microstructure was also measured using cellSens^{TM}. In the damaged SM, one section with the deepest mechanical destruction in hematoxylin-eosin stained sections was selected for histological assessment. The SM was equally divided into shallow and deep layers, and the depth of mechanical destruction was categorized as shallow SM, deep SM, muscularis propria (MP) or deeper. The depth of remaining SM was

defined to be the distance from the point of deepest mechanical destruction to the MP. The width of mechanical destruction was defined as the distance between the two edges of intact mucosa. The depth of remaining SM and width of mechanical destruction were measured. Blood vessels were defined as a tube having both intraluminal red cells and intramural elastic membrane stained in a dark brown color using Elastic Masson stain, and blood vessels measuring 100μm in diameter are referred to as "large vessels". In the area of damaged SM, the number of remaining vessels was counted in Elastic Masson stained sections. Thermal denaturation was also assessed using Elastic Masson stained sections. The SM usually appears green using Elastic Masson stain because of collagen fibers, and thermal denaturation changes collagen fibers into blue whereas mechanical damage does not change the color.

In undamaged SM not affected by surgery, the number and diameter of vessels were counted in Elastic Masson stained sections and compared between shallow and deep SM. Using a microscope (40x), three fields of interest having ≥1 mm thick SM were selected for evaluation. The long axis was defined as the "diameter of the vessel", but a long axis more than twice as long as the orthogonal axis was not considered as the "diameter of the vessel". Instead, the

short axis was defined as the "diameter of the vessel". Vessels located at the intermediate region between shallow and deep layers were not measured.

Outcome Measurements

The primary outcome measure was the depth of mechanical destruction. The secondary outcome measures included the width of destruction, the depth of remaining SM and the number of vessels remaining at the resection sites. The number and diameter of vessels in the undamaged SM were also evaluated.

Sample Size Calculation

The required sample size was determined based on the main outcome measure (depth of destruction) obtained from a previous study [23] and also our experience of reviewing surgical specimens after endoscopic resection. We assumed that the rates of deeper destruction (deeper SM or MP) were 95% after hot snare resection and 50% after cold snare resection. For the study to have 80% power at a significance level of 0.05, 15 patients were required in each arm. Therefore, we recruited 15 patients to meet the inclusion/exclusion criteria and

performed both hot and cold snare resections in each patient.

Statistical Analysis

For nominal data, statistical comparisons were done using the chi-square test. For ordinal or decidedly non-normal data, a Mann Whitney U test for trends was used to compare groups. For decidedly normal data (diameter of vessel), Student's t-test was used for comparison. All p-values are two-tailed, and values <0.05 indicate statistical significance. All statistical analyses were performed with Stata 13.0® (Stata Corp., TX, United States).

Results

Patient

From December 2015 to November 2016, a total of 98 patients underwent colorectal resection for cancer. Of these, 15 patients met the inclusion criteria/exclusion criteria and were prospectively enrolled in this study. All patients were fully evaluated. Patients' gender, age and location of lesions are shown in Table 1.

Evaluation of Submucosal Damage

In the evaluation of mechanical destruction, all cold snare resections were limited to the shallow SM whereas 60% of hot snare resections reached the deep SM and 20% advanced to the MP. The distribution showed a significant deviation ($p<0.001$, Table 2). The rate of deep destruction (deep SM or MP) was significantly higher after hot snare resections (80%), compared with cold snare resections (0%, $p<0.001$). One example is shown in Figures 2-A and 2-B. In all hot snare resections, thermal denaturation (not mechanical destruction), evaluated in Elastic Masson stained sections, occurred through the entire SM and advanced to the MP. The depth of remaining SM after cold snare resections was significantly greater than that after hot snare resections ($p=0.007$, Table 3, Figure 2-A, 2-B). There was no significant difference in the width of destruction between cold and hot snare resections ($p=0.58$, Table 3, Figures 2-A, 2-B). Diminutive vessels were unable to be identified due to resection damage, and large vessels (≥100μm) alone were countable. The median number of remaining large vessels was three after hot snare resections and five after cold snare resections, showing a trend toward fewer after hot snare resections ($p=0.15$,

Table 3, Figure 3-A, 3-B).

Evaluation of Undamaged Submucosa (Table 4, Figure 4)

There were significantly more vessels in the shallow layer identified with intraluminal red cells and intramural elastic membrane compared with the deep layer ($p=0.0015$). Conversely, the number of large vessels (≥100μm) was significantly less in the shallow layer ($p=0.018$). The diameter of vessels was significantly less in the shallow layer, compared with the deep layer ($p<0.001$).

Discussion and Conclusions

This is the first study to compare the histology of cold versus hot endoscopic resections in the colon. This study demonstrates that hot snare resection causes deeper damage than cold snare resection, and frequently reached as deep as the MP. Thermal denaturation permeated through the SM in all specimens resected by hot snare resection. These observations verify that the tissue injury caused by hot snare resection extends to deeper layers than injury after cold snare resection. One previous report showed that thermal

damage in the colon after hot snare resection may cause full-thickness necrosis and perforation [23]. In contrast, mucosal damage in the horizontal plane was almost the same for both hot and cold snare resections, suggesting that an electrical burn occurred just beneath the mucosa constricted by the snare but did not extend horizontally, due to the nature of the high-frequency electric current.

Deeper damage due to use of the electrocautery does not completely explain the underlying mechanism why delayed hemorrhage after cold snare polypectomy occurs less frequently than after hot snare polypectomy. The present study demonstrates that the deep SM contains more large blood vessels. The greater number of large vessels in the deep SM are more severely damaged and subsequently rupture after hot snare polypectomy. As a result of damage to the deeper layer, hot snare resection may lead to delayed bleeding. In contrast, the injury caused by cold snare resection reaches only the shallow SM with no thermal damage. This is less likely to damage large vessels, with a decreased risk of delayed bleeding.

The optimal technique for polypectomy should satisfy many conditions such as safety, curability, feasibility, and ability to be performed rapidly. According to previous reports, the complete resection rate after cold snare

resection reached ≥95% [25-27]. A recent clinical trial also reported that the complete resection rate for cold snare polypectomy (4-9mm) is not inferior to that for hot snare polypectomy [28], although to date, the long-term outcomes of cold snare polypectomy have not been investigated. Cold snare polypectomy was significantly more effective for diminutive polyps and was superior to hot snare polypectomy in terms of procedure time [14, 29]. The use of cold snare polypectomy is increasingly being explored; indications for cold snare polypectomy have expanded to include large (>1cm) colon polyps, which has already being proven to be feasible, safe and effective [29, 30]. A recent report demonstrated that cold snare resection in a piecemeal fashion was safe and effective [31]. Cold snare polypectomy may be useful for the resection of duodenal polyps because duodenal polypectomy is prone to complications due to the thin duodenal wall and abundant vessels [30]. The observations in the present study provide evidence for the expanded indications for cold snare polypectomy.

There were no complications in these fifteen patients (data not shown). Written informed consent was obtained from all patients. The study protocol followed ethical guidelines and was approved by the Institutional Review Board. Patients who participated in this clinical trial were aware that the trial would not

benefit them personally, and that the only potential benefit was to contribute to the advancement of medical science. To obtain statistical significance, therefore, we carefully calculated the sample size, and validated the hypothesis. We recognize the importance of such an elaborate sample size calculation in a clinical trial.

The present study provides detailed information regarding the distribution, number and diameter of vessels in the SM. One expert in the field of endoscopic submucosal dissection inferred that large vessels in the gastrointestinal wall penetrate the MP vertically and then inflow horizontally at the level of the middle SM forming the a ramified vascular network [32]. The observations in the present study reinforce this speculation and provide valuable anatomical knowledge for colonoscopists.

There are acknowledged limitations to this study. First, histological assessment was not blinded to the two reviewers, who recognized which sites as cold or hot. However, the lack of blinding is inevitable in the present study design. Second, we used normal mucosa instead of adenomatous tissue. There could be a difference in the number of vessels, diameter of vessels, tissue resistance and thermal conduction. Normal mucosa close to the cancer was

biopsied as a surrogate polyp. If actual adenomatous tissue was required, this study would not be feasible since two polyps are rarely located next to the cancer. Third, there may be some differences based on location, but we were not able to examine these potential differences due to the limited sample size. Delayed bleeding predominantly occurred in the proximal colon [33]. Future histologic studies may elucidate the underlying reason. Fourth, histological changes were evaluated only one day after endoscopic resection. Greater changes in histology may not have been observed at this time point, and it is noted that the majority of delayed bleeding occurs within three days after polypectomy [34]. Despite these limitations, we believe that this study will have a profound impact on clinical practice because it demonstrates that the cold resection technique can be used in various situations due to its less invasive nature.

In conclusion, hot snare resection results in damage to the deep layers of the colon wall involving more large blood vessels. This may explain the mechanism for the decreased incidence of delayed hemorrhage after cold snare polypectomy.

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Table 1. Patient demographics

Characteristic	n=15
SEX, n (%)	
Female	6 (40.0)
Male	9 (60.0)
Age, years	
median	68
range	48 - 86
Location, n (%)	
Proximal colon	4 (26.7)
Distal colon	5 (33.3)
Rectum	6 (40.0)

proximal colon: cecum, ascending and transverse colon

distal colon: descending and sigmoid colon

Table 2. Depth of destruction

Depth	Cold resection	Hot resection	p-value*
Shallow SM, n(%)	15 (100)	3 (20)	<0.001
Deep SM, n(%)	0	9 (60)	
MP, n(%)	0	3 (20)	
All, n(%)	15 (100)	15 (100)	

SM: submucosa, MP: muscularis propria

The submucosa was equally divided into shallow layer and deep layer.

* chi-square test

Table 3. Evaluation of Submucosal Damage.

Factor	Cold resection		Hot resection		p-value*
	Median	IQR,	Median	IQR	
Depth of remaining SM, μm	711	296 - 1583	187	68 - 774	0.007
Width of damaged SM, μm	3086	2583 - 5141	3422	2466 - 6938	0.58
Remaining large vessels, n	5	2 - 6	3	0 - 5	0.15

SM: submucosa, IQR: interquartile range

Depth of remaining SM: the distance from the deepest destruction to the muscularis propria

Width of damaged SM: the distance between both edges of intact mucosa

Remaining large vessels: the number of blood vessels ≥100μm counted in the area of damaged SM.

*Wilcoxon signed-rank test

Table 4. Blood Vessels in Undamaged Submucosa

Factor	Shallow SM		Deep SM		p-value
	Median	IQR	Median	IQR	
Number of vessels, n	50	31 - 60	29	19 - 40	0.0015*
Number of large vessels, n	4.3	2 - 5	7.6	5 - 9	0.018*
	Mean	SD	Mean	SD	
Diameter of vessels, μm	44.6	37.1	78.3	66.5	<0.001**

SM: submucosa, IQR: interquartile range, SD: standard deviation

*Wilcoxon signed-rank test, **Student t test

738 vessels in shallow SM and 444 vessels in deep SM were evaluated.

Figure

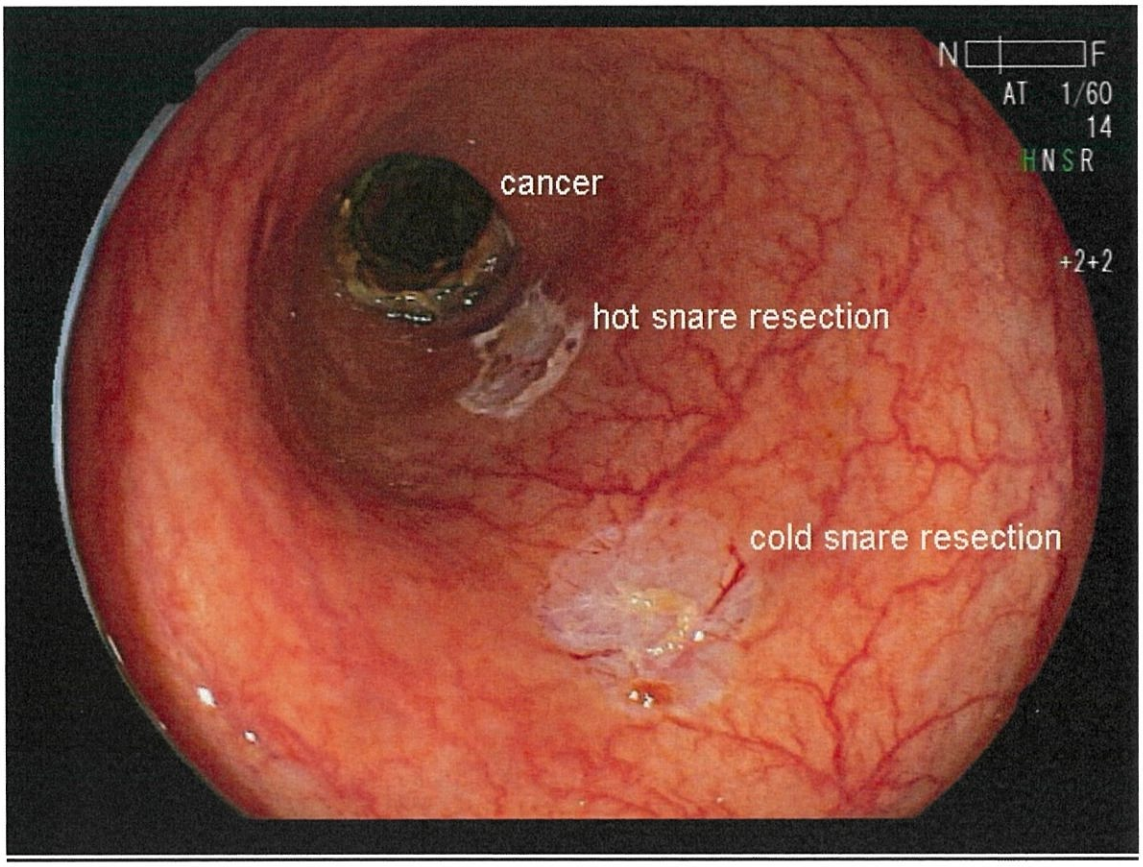

Figure 1. Endoscopic resection sites were selected close to the distal side of the cancer to be easily included in the surgical specimens. The resection sites were aligned longitudinally in the bowel lumen.

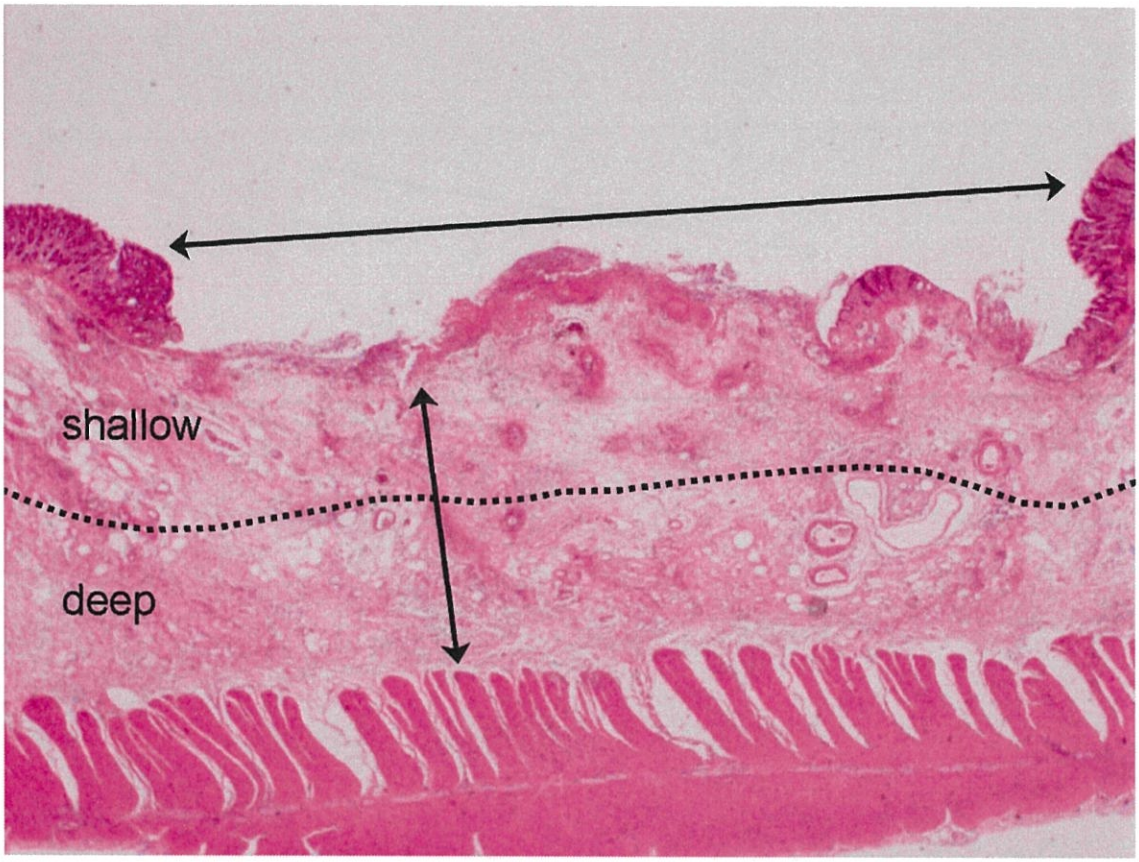

Figure 2-A. Cold snare resection. The depth of destruction was evaluated using hematoxylin-eosin (40X). The submucosa was divided equally into shallow and deep layers (dotted line). The cold snare resection was limited to the shallow submucosa. The width of destruction (indicated by the horizontal line with two arrowheads) was 3322μm. The depth of remaining submucosa (indicated by a vertical line with two arrowheads) was 2082μm.

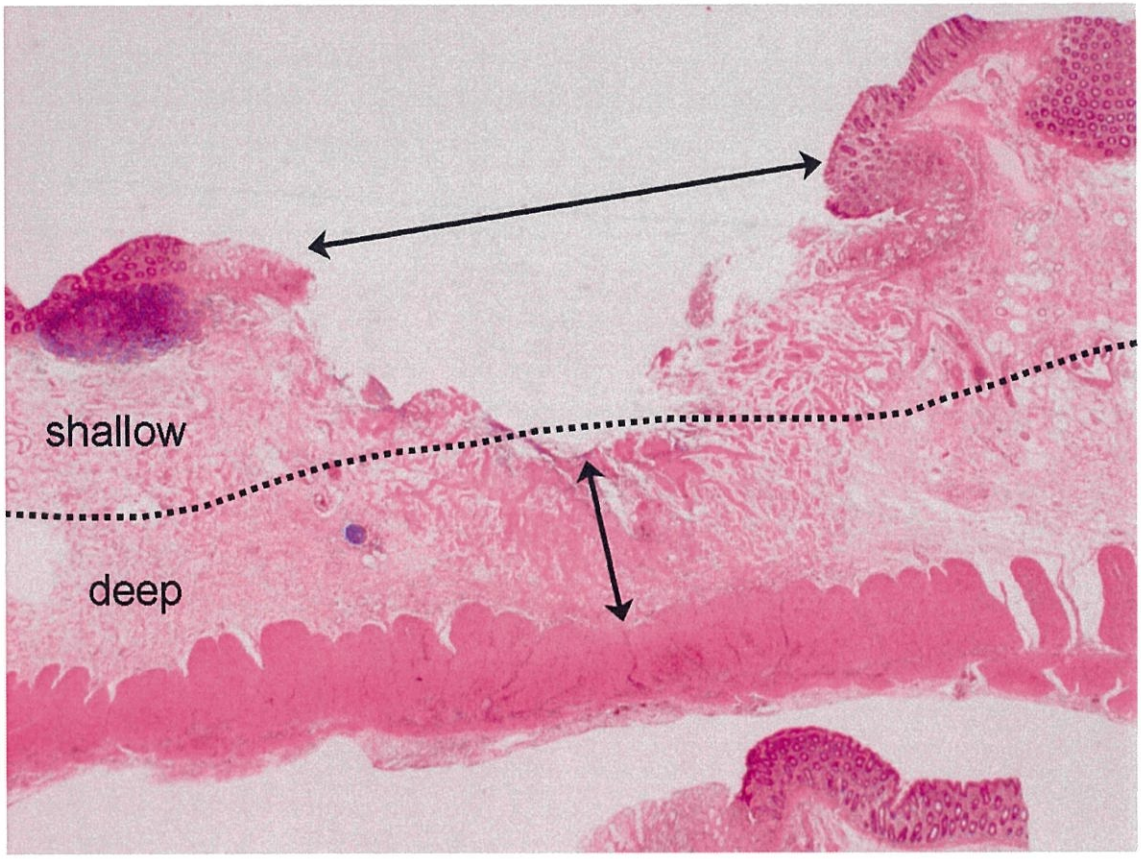

Figure 2-B. Hot snare resection. The hot resection reached the deep submucosa adjacent to the muscularis propria. The width of destruction was 2417μm and the depth of remaining submucosa was 794μm.

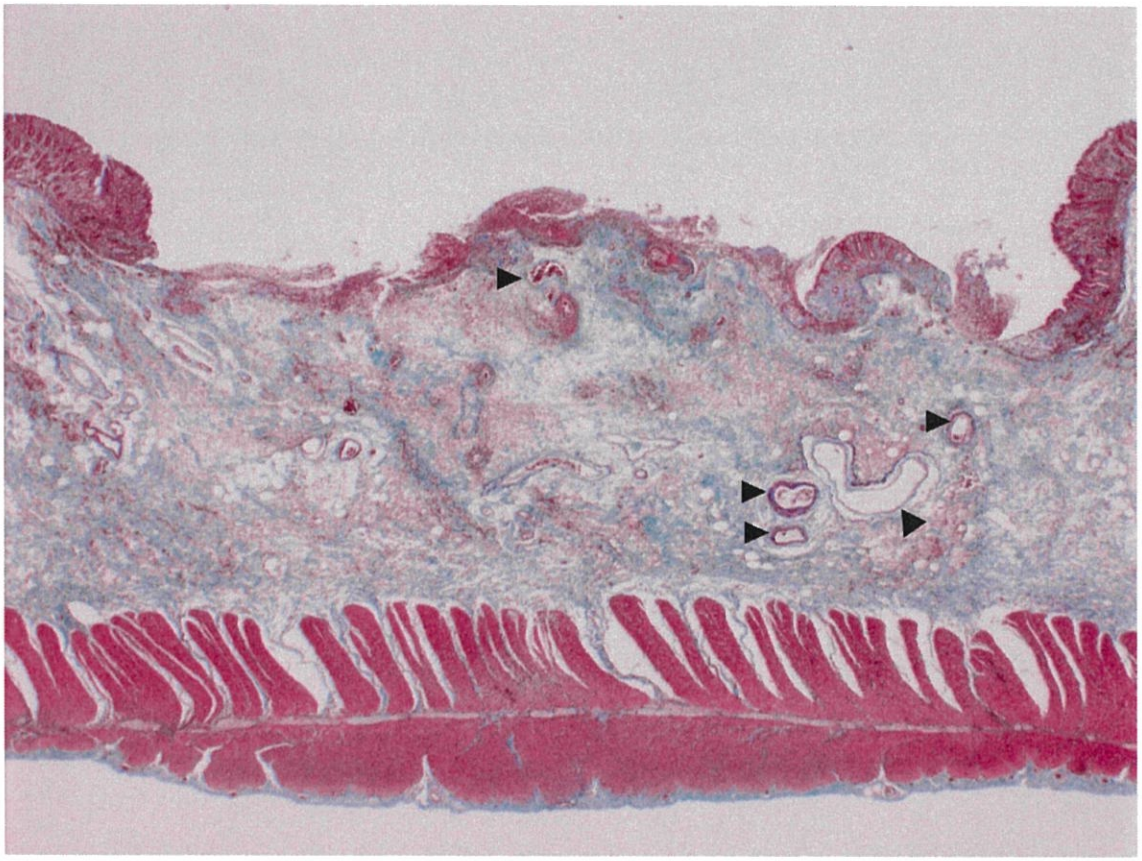

Figure 3-A. Cold snare resection. The number of remaining large vessels (>100μm, arrowhead) was counted using Elastic Masson stain (40x). A blood vessel was defined as a tube having both intraluminal red cells and an intramural elastic membrane stained dark brown. The number of remaining submucosal vessels in this specimen was five, after cold snare resection.

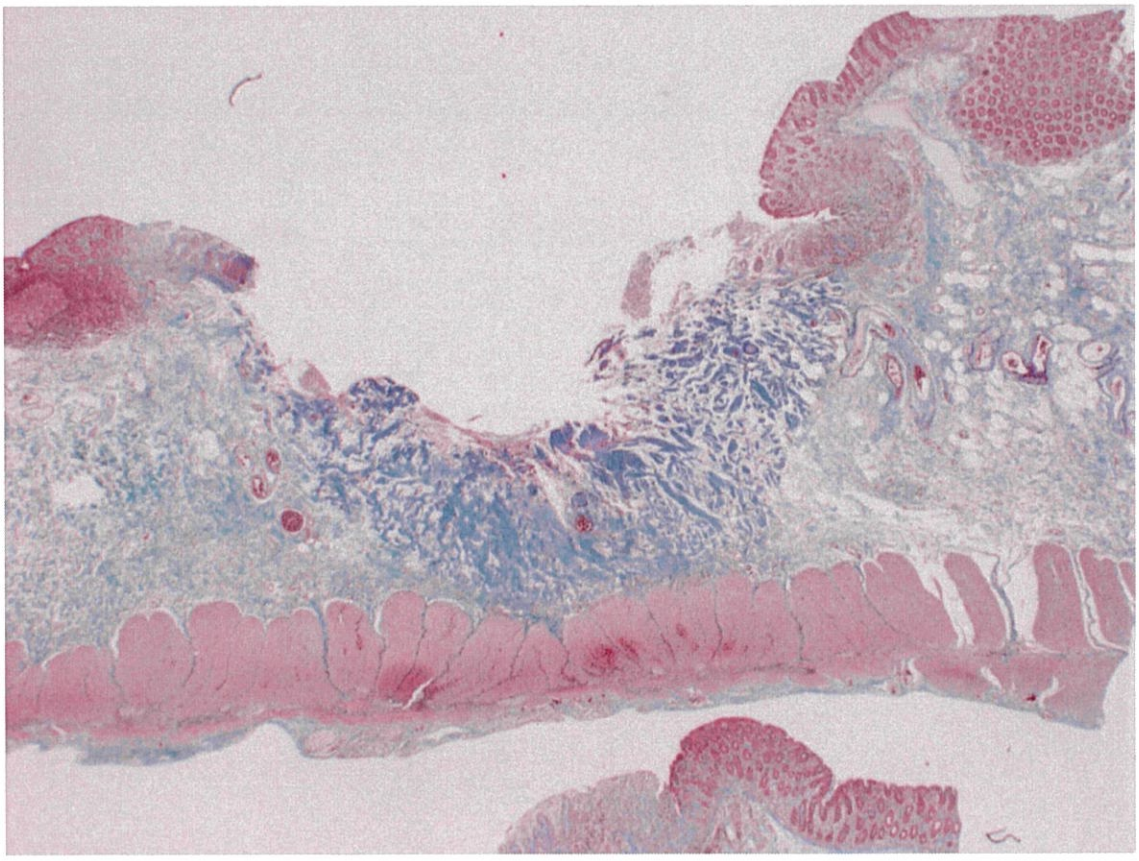

Figure 3-B. Hot snare resection. Collagen fibers changed to a blue color by thermal injury were present throughout the submucosa. No remaining large vessels were observed in this specimen.

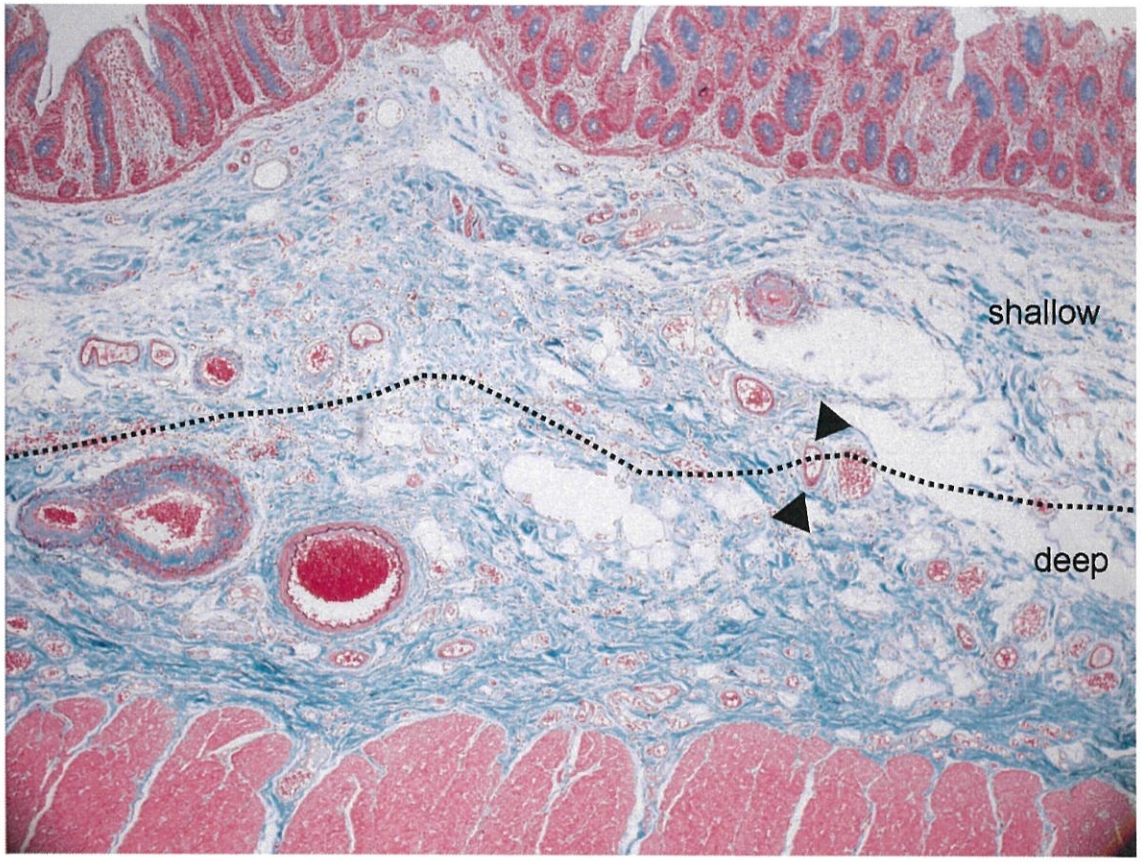

Figure 4. Using Elastic Masson stain (40X), submucosa ≥1 mm thick was selected and divided equally into shallow and deep layers. Vessels (arrowheads) located in the intermediate region between shallow and deep layers were not evaluated. The vascular diameter is larger in the deep submucosa, compared with the shallow submucosa by visual inspection.